A/DAC

Please type a plus sign (+) inside this box → [+]

PTO/SB/05 (4/98)
Approved for use through 09/30/2000. OMB 0651-0032
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No.	
First Inventor or Application Identifier	Kenneth E. Sherman
Title	Composition and Method of Treating Hepatitis C
Express Mail Label No.	

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents, Box Patent Application, Washington, DC 20231

1. [X] * Fee Transmittal Form *(e.g., PTO/SB/17)* *(Submit an original and a duplicate for fee processing)*
2. [X] Specification [Total Pages 21]
   (preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross References to Related Applications
   - Statement Regarding Fed sponsored R & D
   - Reference to Microfiche Appendix
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings *(if filed)*
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure
3. [ ] Drawing(s) *(35 U.S.C. 113)* [Total Sheets]
4. Oath or Declaration [Total Pages]
   a. [ ] Newly executed (original or copy)
   b. [X] Copy from a prior application (37 C.F.R. § 1.63(d)) *(for continuation/divisional with Box 16 completed)*
      i. [ ] DELETION OF INVENTOR(S) Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

*** NOTE FOR ITEMS 1 & 13: IN ORDER TO BE ENTITLED TO PAY SMALL ENTITY FEES, A SMALL ENTITY STATEMENT IS REQUIRED (37 C.F.R. § 1.27), EXCEPT IF ONE FILED IN A PRIOR APPLICATION IS RELIED UPON (37 C.F.R. § 1.28).**

5. [ ] Microfiche Computer Program *(Appendix)*
6. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
   a. [ ] Computer Readable Copy
   b. [ ] Paper Copy (identical to computer copy)
   c. [ ] Statement verifying identity of above copies

OIPE JC35 APR 0 6 2000 PATENT & TRADEMARK OFFICE

ACCOMPANYING APPLICATION PARTS

7. [ ] Assignment Papers (cover sheet & document(s))
8. [ ] 37 C.F.R.§3.73(b) Statement *(when there is an assignee)* [ ] Power of Attorney
9. [ ] English Translation Document *(if applicable)*
10. [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations
11. [ ] Preliminary Amendment
12. [ ] Return Receipt Postcard (MPEP 503) *(Should be specifically itemized)*
13. [ ] * Small Entity Statement(s) *(PTO/SB/09-12)* [ ] Statement filed in prior application, Status still proper and desired
14. [ ] Certified Copy of Priority Document(s) *(if foreign priority is claimed)*
15. [ ] Other:

16. If a **CONTINUING APPLICATION**, *check appropriate box, and supply the requisite information below and in a preliminary amendment:*

[X] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application No: 08 / 844,349

Prior application information: Examiner Jay Williams Group / Art Unit: 1643

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. CORRESPONDENCE ADDRESS

[ ] *Customer Number or Bar Code Label* *(Insert Customer No. or Attach bar code label here)* or [X] *Correspondence address below*

Name	Maria H. Sjogren, M.D., MC, Col. USA				
	Chief, Department of Clinical Investigation				
Address	Walter Reed Army Medical Center, Bldg. 6, Rm 4018				
	6900 Georgia Avenue, N.W.				
City	Washington	*State*	D.C.	*Zip Code*	20307
Country	USA	*Telephone*	(202) 782-7859	*Fax*	(202) 782-3881

Name (Print/Type)	Werten F.W. Bellamy	*Registration No. (Attorney/Agent)*	27,029
Signature	Werten F.W. Bellamy	*Date*	4/5/00

COMPOSITION AND METHOD OF TREATING HEPATITIS C

I. GOVERNMENT INTEREST

This invention described herein may be manufactured, used and licensed by or for the Government for governmental purposes without the payment to us of any royalties thereon.

II. RELATED APPLICATION

This application is a continuation-in-part of U.S. Patent Application Serial No. 08/404,844 filed January 24, 1994, which is a continuation of U.S. Patent Application Serial No. 07/878,372 filed May 4, 1992 which in turn is a continuation in part of U.S. Patent Application Serial No. 07/759,544, filed September 13, 1991.

III. FIELD OF INVENTION

This invention relates generally to the pharmacological treatment of hepatitis C virus infection in patients.

IV. DESCRIPTION OF THE RELATED ART

Hepatitis C Virus (HCV), the putative agent in the majority of post-transfusion acquired hepatitis, has been recently defined by a new serologic assay. Kuo, G., et al., Science, 244:362-4 (1989). Despite improvement in the quality of the blood-donor pool and the recent implementation of testing of donated blood, the current estimated incidence of acute infection

among persons receiving transfusions is 5 to 10%. Alter, H.J., in Zuckerman, A.J., ed., Viral Hepatitis and Liver Disease, Allen K. Liss, New York, 1988, pp.537-42. Chronic hepatitis develops in at least half the patients with acute HCV infection (representing about 90% of patients with non-A, non-B hepatitis (NANB)), and cirrhosis develops in at least 20% of this group. Thus, of the approximately 3 million persons who receive transfusions in the United States each year, acute hepatitis C will develop in about 150,000. Chronic hepatitis C will develop in at least 75,000 of these, and among them cirrhosis will develop in more than 15,000. Among patients with post-transfusion hepatitis, up to about 90% are positive for the HCV antibody. Davis, G.L., et al., New England Journal of Medicine, 321:1501-6 (1989). Patients with sporadic NANB hepatitis (no specific risk factors) are also very likely to have the anti-HCV antibody. Kuo, et al. (1989) above. While most of the patients who contract hepatitis C will have subclinical or mild disease, approximately 50% will progress to a chronic disease state characterized by fluctuating serum transaminase abnormalities and inflammatory lesions on liver biopsy. By some estimates, cirrhosis will develop in up to about 20% of this group. Koretz, R.L., et al., Gastroenterology, 88:1251-4 (1985).

With the aim of halting or slowing the progression of HCV-related diseases, a variety of drugs have been evaluated in recent years. Both acyclovir and corticosteroids (which are beneficial in autoimmune chronic active hepatitis) are ineffective. Pappas, S.C., J. Med. Virol., 15:1-9 (1985); Stokes, P., et al., Gastroenterology, 92:1783 abstract (1987).

To date, α-interferon (IFA) appears to be the most promising candidate, although not necessarily the final answer. Hoofnagle, J.H., et al., in Viral Hepatitis: 1981 International Symposium, Philadelphia, Franklin Institute Press, 1982, pp. 573-83; Hoofnagle, J.H., et al., New England Journal of Medicine, 315:1575-8 (1986); Thomson, J., Lancet, 1:539-41 (1987); Kiyosawa, K., et al., in Zuckerman, A., ed., Viral Hepatitis and Liver Disease, Allen K. Liss, New York, 1983, pp. 895-7. Hoofnagle, J.H., et al., Sem. Liver dis., 9:259-263 (1985). The interferons are host proteins made in response to viral infections as well as other antigenic stimuli. They are classified by their cell or origin as well as their antigenicity. α-Interferon is made by lymphoblastoid cells, β-interferon by fibroblasts, and γ-interferon by T-cells. Subtypes in each group are based on antigenic/structural characteristics. Recombinant forms for each group have been developed and are commercially available. A pilot study

utilizing IFA on ten patients with well-characterized post-transfusion NANB hepatitis was reported in 1986 by Hoofnagle et al. (Hoofnagle, J.H., et al., New England Journal of Medicine, 315:1575-8 (1986)). In this study, eight of ten patients improved their serum alanine transaminase (ALT) levels within one month of starting therapy. IFA therapy consisted of 5 million units (MU) daily in seven of the patients and one MU daily in three patients. In all subjects the dose was gradually reduced to 1 MU daily and then finally switched to an alternate day or every three day regimen. In three patients who had post-treatment liver biopsies, the specimen showed a marked improvement in the degree of portal inflammation and loss of parenchymal hepatocytic necrosis. Side effects were common at the 5 MU/day dose and virtually absent at 1 MU/day.

The effects of recombinant human interferon α in a prospective, randomized, double-blind, placebo-controlled trial in patients with well-documented chronic HCV infection has recently been carried out. Di Bisceglie, A.M., et al., New England Journal of Medicine, 321:1506-10 (1989). Forty-one patients were enrolled in the trial, 37 of whom were later found to have antibody to HCV. Twenty-one patients received interferon α (2 MU) subcutaneously three times weekly

for six months, and twenty received placebo. The mean serum ALT and the histological reatures of the liver improved significantly in the patients treated with interferon, but not in the patients given placebo. Ten patients treated with interferon (48%) has a complete response, defined as a decline in mean serum ALT to the normal range during therapy; three others had a decrease in mean ALT of more than 50%. After treatment ended, however, serum ALT usually returned to pretreatment levels; six to twelve months after the discontinuation of interferon therapy, only two patients (10%) still had normal values. The authors concluded that interferon α therapy is beneficial in reducing disease activity in chronic hepatitis C; however, the beneficial responses are often transient and side effects are known to appear.

In another, broader study, chronic hepatitis C (NANB hepatitis) is 166 patients was treated with either 3 MU or 1 MU of recombinant human α-IFA three times weekly for 24 weeks or to no treatment. The serum ALT level became completely normal in 22 of the 26 patients (85%) who responded to treatment with 3 MU of interferon, and nine of the sixteen patients (56%) responded to treatment with 1 MU. The patients who received 3 MU of interferon had histologic improvement because of the regression of lobular and periportal

inflammation. However, relapse within six months after the completion of treatment occurred in 51% of the patients treated with 3 MU of interferon and in 44% of those treated with 1 MU. Davis, G.L., et al., New England Journal of Medicine, 321:1501-06 (1989). These authors concluded that a 24-week course of interferon therapya is effective in controlling disease activity in many patients with hepatitis C, although relapse after the cessation of treatment is common.

A multi-center randomized control trial of recombinant human α-IFN in patients with chronic NANB hepatitis has been reported recently. Marcellin, P., et al., Hepatology, 13:393-97 (1991). Patients were randomly assigned to no treatment or to 1 to 3 MU of α-interferon given three times a week for 24 weeks. Forty-five patients (75%) were positive for antibody to HCV. During the 24 week treatment period, mean serum ALT levels decreased in both treatment gropus, but the decrease was statistically significant only in the 3 MU group. However, at 24 weeks, the proportion of patients with normal ALT levels was similar in the 3 MU group (39%) and the 1 MU group (45%) and both were significantly higher than in controls (0%). Repeat lever biopsy specimens showed a significant decrease in the severity of histological changes in the higher dose group but not in the lower dose group or in controls.

However, after treatment, the mean ALT levels rose in both treated groups. The proportion of patients with normal ALT levels at week 48 was 28% in the 3 MU group and 20% in the 1 MU group. The authors conclude that a dose of 3 MU was superior to 1 MU of α-interferon given three times per week for 24 weeks in inducing improvements in serum ALT levels and liver histological examinations. However, relapse in disease activity occurred in approximately half of the responders when interferon was stopped. The response to α-interferon did not correlate with the source of infection or withthe presence or absence of anti-HCV antibody titres in patient sera.

It is clear, therefore, that while α-interferon has a beneficial effect on the course of HCV infection, this effect is frequently only transient. therefore, new modalities are necessary in order permanently to eradicate the effects of hepatitis C virus on the patient.

Another class of polypeptide immune modifiers derived from the thymus gland, the thymosins, has been shown to trigger maturational events in lymphocytes, to augment T-cell function and to promote reconstitution of immune defects. Low, T.L.K., et al., "Thymosins: Structure, Function and Therapeutic Application", Thymus, 6:27-42 (1984).

Thymosin Fraction Five (TF-5), originally described by Goldstein et al. (Proc. Nat'l Acad. Sci. (USA), 69:1800-1803 (1972)), is a partially purified extract of bovine thymus containing at least 40 peptide components, 20 of which have been purified to homogeneity or near homogeneity; it contains about 0.6% of Thymosin α-1 (THNα_1). Low, 1984, above.

THNα_1, initially isolated from TF-5, has been sequenced and chemically synthesized. Wetzel, R., et al., Biochemistry, 19:6096-6104 (1980). Its sequence is highly homologous in mice, calves and humans. THNα_1 is a 28 amino acidic polypeptide with a molecular weight of 3100 that has shown activity qualitatively similar to TF-5 in modulating the immune system. Low, T.L.K., et al., J. Biol. Chem., 254:981-6 (1979). THNα_1 has potent immunologic activity, including stimulation of α- and γ-interferon production, increasing macrophage migration inhibitory factor production, inducing expression of T-cell markers, including IL-2 receptors, and improving T-cell helper cell activity. Schulor, R.S., et al., in The Lynphocyte, Allen J. Liss Inc., New York, 1981, pp. 191-215; Low, T.L.K., et al., in "Thymosins: Structure, Function and Therapeutic Applications", Thymus, 6:27-43 (1984); Koutab, N.M., et al., Immunopharm., 16:97-105 (1988). Studies in mice have demonstrated a

synergistic effect of THNα_1 and interferon on natural killer-cell activity in immunosuppressed mice. Favilli, C., et al., Cancer Immunol. Immunother., 20:189-92 (1985). TF-5 and THNα_1 can influence immunoregulatory T-cell function, promote production of interferon-α, interferon-γ and interleukin-2 by human lymphocytes and increase interleukin-2 receptor expression. Marshall, G. D., et al., J. Immunol., 126:741-4 (1981); Mutchnick, M.G., et al., Clin. Immunol. Immunopathol., 23:626-33 (1982); Sztein, M.B., et al., Proc. Nat's Acad. Sci. (USA), 83:6107-6111 (9186); Serrate, S.A., et al., J. Immunol., 1939:2338-43 (1987); Bazevanis, C.N., et al., Immunopharm., 13:133-41 (9187); and, Svedersky, L.P., Eur. J. Immunol., 12:244-7 (1982).

Clinical trails of TF-5 and THNα_1 as primary or adjunctive therapy in patients iwth immunodeficiency or cancer indicate that these agents enhance immune responsiveness and augment specific lymphocyte functions. Clinical trials of TF-5 and purified THNα_1 have been underway for a number of years. Early trials in patients with cancer or immunodeficiency states were encouraging, though not definitive. Goldstein, A.L., et al., Transp. Proc., 9:1141 (1977); Barrett, D.J., et al., J. Pediatr., 97:61 (1980); and Cohen, M.H., et al., J. Amer. Med. Assoc., 241:1813-5 (1979). THNα_1

use has been described in a randomized trial of patients with nonsmall cell lung cancer. Patients were treated with THNα_1 at a dose of 900 μgrams/m^2 subcutaneously twice weekly or daily for two weeks and then twice weekly after completing a course of radiotherapy. The only side effect of THNα_1 was mild burning at the injection site in three patients. This was attributed to the drug lot and may have been due to the carrier preparation. Relapse-free survival and overall survival were greater in both THNα_1 treatment groups than in the placebo group; some restoration of radiation-suppressed immune function was also noticed. There was no increase in T-cell numbers associated with this. Schulof, R.S., et al., J. Biol. Response Modifiers, 4:147-58 (1985).

Recent double-blind, randomized trials with thymosins have been performed in elderly men in an effort to increase response to influenze vaccine. Gravenstein, S., et al., JAGS, 37:1-8 (1989). Patients received synthetic THNα_1 subcutaneously twice weekly starting at the time the influenze vaccine was given. At six weeks post-vaccine, those patients randomized to receive the drug had higher levels of antibody to influenze than controls. This difference was accentuated in the very elderly (ages 77-99). No

clinical or biochemical toxicity was observed in drug recipients.

There are preliminary reports that thymosins may be effective against infections caused by hepatitis viruses other than HCV. In an animal model of viral hepatitis, the woodchuck infected with the Woodchuck Hepatitis Virus, THNα_1 suppressed viral DNA replication, but produced no improvement in clinical parameters. Korba, B.E., et al., Hepatology, 12:Abs. 880 (1990). In aa pilot clinical trial with patients with Chronic Active Hepatitis B caused by the hepatitis B virus (HBV), patients treated for a year with THNα_1 (5 patients) or with TF-5 (2 patients) showed a marked decrease in serum ALT; 6 of the 7 patients also showed reduced levels of serum HBV DNA, and 5 of 6 patients initially positive for serum hepatitis B surface antigen (HBsAg) subsequently cleared this antigen. Mutchnick, M.C., et al., Hepatology, 10:Abs. 575 (1989). No suggestion was made in these abstracts that the thymosins would be effective against any other hepatitis viruses.

There remains, therefore, an important need in the art for a new modality for the treatment of HCV infections in mammals; this modality is disclosed below.

SUMMARY OF THE INVENTION

A treatment modality for HCV infections has been devised comprising the administration to mammals of immune system-potentiating doses of one or more thymosins in combination with interferon therapy.

It is thus an object of this specification to disclose compositions and methods for the treatment of acute or chronic HCV infections in mammals comprising combination therapy with one or more thymosins and one or more interferons.

This and other objects will become apparent by reference to the specification and to the appended claims.

DESCRIPTION OF THE INVENTION

A novel modality for treating HCV infection in mammals has been devised, comprising the administration to such mammals of one or more thymosins at doses which potentiate immune responses, in combination with anti-viral doses of one or more interferons.

By the term "thymosins" is meant any or all of the immune system potentiating polypeptides naturally occurring in the thymus gland or produced by chemical or recombinant means, or fragments derived from any of these polypeptides. By the term "mammals" is meant any mammalian subject, including human and animal patients, requiring treatment for hepatitis C infection. "Mammal" and "subject" are used interchangeably.

Thymosin preparations suitable for treating HCV infections include TF-5, $THN\alpha_1$ and fragments thereof, e.g., C-terminal 4-28 and 15-28, and N-terminal 1-8, 1-14 and 1-20 fragments. These may be obtained from Alpha-1 Biomedicals Inc., Foster City, California.

Subjects, e.g., human patients, may receive the thymosin by subcutaneous injection or infusion, at appropriate intervals for an appropriate period of time. The thymosin is administered to mammals infected with hepatitis C virus in amounts which facilitate or promote in vivo inactivation of hepatitis C virus. A pharmaceutical dosage unit of an immune system-potentiating amount of a thymosin, such as TF-5, can be from about 900 to about 1200 mg/m^2 body surface area in a pharmaceutically acceptable carrier. A pharmaceutical dosage unit of an immune system-potentiating amount of a thymosin, such as $THN\alpha_1$ or immune system-potentiating fragments thereof, can be from about 900 to about 1200 $\mu g/m^2$ body surface area in a pharmaceutically-acceptable carrier. Lyophilized preparations of thymosins or fragments which contain mannitol and phosphate buffer are dissolved in diluent period to dispensing. Thymosins in diluent should remain stable for at least six months when stored in a refrigerator. It is convenient to dispense thymosin solutions in one ml dose vials per month.

For a thpical human patient, an administration regimen of twice weekly (e.g., Monday and Thursday) subcutaneous injection of about 1500 to about 1700 μg of THNα_1 or fragments therefrom is convenient. Dosages and length of treatment can be flexible, and can be determined by the subject's clinical response to the thymosins.

The course of the disease and its response to drug treatments may be followed by clinical examination and laboratory findings. As elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are known to occur in uncontrolled hepatitis C, and as a complete response to treatment is generally defined as the normalization of these serum enzymes, particularly ALT (Davis, G.L., et al., New England Journal of Medicine, 321:1501-6 (1989)), progress of treatment with thymosins is conveniently followed by this art-recognized test performed, e.g., on a sequential multiple analyzer.

Another means of evaluating subjects having antibodies to HCV (not all subjects with hepatitis C have detectable antibody to HCV - Weiner, A.J., et al., Lancet, 335:1-3 (1990)) is to periodically test subjects' sera for the titer of these antibodies. Anti-HCV antibodies may be tested by the currently available C 100-3 test (Kuo, G., et al., Science,

244:362-4 (1989)), by an Elisa test (Ortho Diagnostic Systems, Raritan, N.J.) or by a recombinant assay (RIBA-1 and RIBA-2, Chiron Corporation, Emeryville, CA). Any suitable test may be used.

In order to follow the course of HCV replication in subjects in response to drug treatment, HCV RNA may be measured in serum samples by, for example, a nested polymerase chain reaction assay that uses two sets of primers derived from the NS3 and NS4 non-structural gene regions of the HCV genome. Farci, P., et al., <u>New England Journal of Medicine</u>, 325:98-104 (1991); Ulrich, P.P., et al., <u>J. Clin. Invest.</u>, 86:1609-14 (1990).

Other appropriate laboratory tests to follow the course of treatment oare listed in Example 1 below.

thymosin therapy is preferably used in combination with interferon therapy, therby combining the immune system potentiating effect of thymosins with the anti-viral effects of the interferons. An improved response rate at the currently used interferon doses would be beneficial, particularly in the light of dose-limiting side effects at higher doses of these proteins. An offshoot ofthis concept is the ability to achieve comparable efficacy with interferon plus thymosin at lower doses than would be required with interferon alone.

In this combination therapy regimen, one or more interferons (for example, recombinant interferon α-2b, Intron-A, Schering-Plough, Kenilworth, New Jersey) is (are) administered subcutaneously to subjects, e.g., human patients, at doses ranging between about 1 MU and 3 MU along with or sequentially with one or more thymosins, preferably including THNα_1, at a dose of about 900 to about 1200 $\mu g/m^2$ body surface area.

Although the example above speaks in terms of recombinant interferon α-2b, other anti-HCV-effective interferons such as α-, β- and γ-interferons, recombinant or naturally occurring, may be advantageously used in this invention.

This combination dose regimen is flexible, and depends on the clinical condition of the subject. Where subjects are refractory to the preferred dosage levels, these may be increased within the limits dictated by undesirable side effects. Typically, injections are made five times per week and continue until an acceptable response by the subject is realized.

Tests to determine the effectiveness of the combination therapy may be the same as those described above for thymosin treatment alone. In addition, histological examination of liver biopsy samples may be used as a second major criteria for evaluation.

Knodell, R.G., et al., Hepatology, 1:431-5 (1981), whose Histological Activity Index (portal inflammation, piecemeal or bridging necrosis, lobular injury and fibrosis) provides a scoring method for disease activity.

The following examples are provided merely to illustrate the invention, and are not to be construed in any way as limiting the scope of invention as set forth in the specification and claims.

EXAMPLE 1

Preparation of Injectable Formulation

Pharmaceutical dosage units or 1 ml each are prepared from the ingredients shown in Table 1 below.

TABLE 1

Active Ingredient	Amount Per mL
Thymosin α-1	0.0016 g
Inactive Ingredients	
mannitol, U.S.P.	0.050 g
sodium phosphate dibasic, heptahydrate, U.S.P.	0.002 g
sodium phosphate monobasic, monohydrate, U.S.P.	0.0005 g
sodium phosphate dibasic, 2 mg/ml solution	
sodium phosphate monobasic, 0.5 mg/ml solution	
water for injection, U.S.P.	

EXAMPLE 2

Treatment of Hepatitis C Infections in Human Patients with Thymosins and Interferons

Adult patients with chronic active hepatitis C (CAHC) are randomized to one of four study groups, made up of about 40 patients per group. Selection criteria include: (1) patients are adults (at least 18 years of age); (2) serum ALT is elevated for at least six months prior to treatment iwth at least one value greater than twice the upper limit of normal in the laboratory doing the testing; (3) patients test positive for HCV antibody on two occasions andon a confirmatory test; and (4) liver biopsy within three months of treatment exhibits pathology consistent with chronic active hepatitis.

Exclusion criteria include: (1) recent use of other anti-viral or immunosuppressive medication; (2) hemophilia, pregnancy or HIV invection, or other serious illness that could prevent completion of the course of treatment; (3) other forms of liver disease, including hepatitis A or B, α-1 antitrypsin deficiency, Wilson's disease, and hemochromatosis must be absent; (4) autoimmune markers (ANA, ASMA, AMA, anti-LKMI) must be absent or, if present, titers should be < 1:40; (5) leukocyte deficiency (<3,000); (6) low absolute neutrophil count (<1,000); (7) low platelets (<75,000);

(8) low Hb (<11 g/dL); (9) high bilirubin (>4 mg/dL); and (10) low serum albumin (3 g/dL).

The first of the four randomized groups receives interferon, preferably interferon α-2b, at a dose of 3 million units (MU) subcutaneously (SQ) on Mondays, Wednesdays and Firdays, and receives placebos on Tuesdays and Saturdays. The second group receives the same dose/schedule of interferon, plus a thymosin, preferably THNα_1, at a dose of 900 $\mu g/m^2$ SQ on Tuesdays and Saturdays. The third group receives the same dose/schedule of a thymosin alone. The fourth group receives placebo treatment initially, but can be randomized to the three treatment groups thereafter. Interferons and thymosins can be recombinant.

Patients begin treatment while hospitalized for about one week, during which period side-effects are monitored.

Outpatient follow-up is initially at one week intervals for two weeks, then at two seek intervals for two months, and then monthly for the remainder of the treatment period. At each visit the following lab tests are performed: CBC, platelet count, differential and ESR, ALT, AST, GGT, alkaline phosphatase, bilirubin, total bilirubin/albumin and HCV antibody. At monthly intervals serum γ-globulin, TSH, ANA and ASMA are assessed.

Drug toxicity is monitored on an ongoing basis using both clinical and laboratory parameters.

Wtihin one month of completing the initial six months of treatment, patients undergo liver biopsy for pathological examination according to Knodell et al. above. This system provides a numerical scoring system of histological activity in patients with asymptomatic CAH.

At this time, control patients are randomized into three groups to receive one of the three treatment modalities, assuming that they still have CAH on follow-up liver biopsy, and that one arm of the study does not show highly significant positive or negative results on analysis at six months.

Patyients in the treatment groups are followed to evaluate recrudescense of disease as evidenced by rising ALT levels. Patients who showed response in the initial six month treatment period, but who have a recurrence of the disease therafter, are provided with additional therapy.

Additional serum or tissue tests are performed if possible: evaluation of antibodies to interferons and thymosins, polymerase chain reaction amplification of hepatitis C genome segments in liver biopsy samples, and quantitative evaluation of anti-hepatitis C serum titers.

EXAMPLE 3

The treatment protocol is as in Example 2, except that the interferon is used at the level of 2 MU, and the thymosin at 1050 $\mu g/m^2$.

EXAMPLE 4

The treatment protocol is as in Example 3, except that 1 MU of the interferon and 1200 $\mu g/m^2$ of the thymosin are used.

EXAMPLE 5

Analysis of Data

There are two primary criteria for response to therapy- normalization of ALT levels by the end of the treatment period (a partial response may be difined as a decrease of at least 50% of initial ALT), and histological improvement as determined by the Histological Activity Index (HAI) of Knodell et al. above.

This analysis provides a raw score ranging from 1 to 22 per sample. Paired data can be analyzed using the Wilcoxon paired-sample test. Additionally, samples can be classified into mild, moderate or reverse CAH, and improvement assessed using the Chi-square statistical analysis.

Life-table analysis is used to evaluate remission and relapse status in terms of normalization of ALT levels. Other continuous variables are analyzed using

Student's t test. Dichotomous data are subjected to CHi square of Fisher's exact test, as is appropriate.

A power analysis was done to determine the number of patients in each test group in order to show predicted differences. Power analysis applied to an ANOVA using a power of 0.80 with $\alpha = 0.05$, coupled with prior studies of mean ALT levels and their variances, estimated a need for 21 to 52 patients in each test group to show a mean ALT difference of 15 IU/L. As 3 to 5% of patients are expected to drop out, and factoring in treatment of the control group after six months, 40 patients per group was arrived at.

We Claim:

1. A method of treating a mammal infected with hepatitis C virus, comprising administering to said mammal an anti-viral effective amount of at least one interferon, concurrently or sequentially with administering said thymosin or thymosin fragment.

2. A method of Claim 1, wherein said interferon is selected from the group consisting of α-, β- and γ-interferons.

3. A method of Claim 2, wherein said α-interferon is interferon α-2b.

4. A method of Claim 1, wherein the step of administering said interferon comprises administering interferon produced by recombinant DNA technology.

5. A method of Claim 1, wherein said mammal is a human, said interferon is an α-interferon, and the amount of said interferon administered ranges between about one million and about three million units of said interferon per administration.

6. The method of Claim 1, wherein said mammal is human, said thymosin is thymosin α-1, and said dose is about 1500 to about 1700 μg of said thymosin α-1.

7. A composition comprising a pharmaceutical dosage unit of a pharmaceutically acceptable carrier containing an immune system-potentiating amount of at least one member selected from the group consisting of thymosin and immune system-potentiating fragments of thymosin in combination with an anti-viral effective amount of at least one interferon, said pharmaceutical dosage unit being capable of promoting <u>in vivo</u> inactivation of hepatitis C virus when administered to mammals infected with said virus.

8. A composition of Claim 7, wherein said thymosin is selected from the group consisting of Thymosin Fraction Five and Thymosin α-1.

9. A composition of Claim 7, wherein said interferon is selected from the group consisting of α-, β-, and γ-interferons.

10. A composition of Claim 9, wherein said α-interferon is interferon α-2b.

11. A composition of Claim 10, wherein said interferon is recombinant interferon.

12. The composition of Claim 7, wherein said thymosin is Thymosin Fraction Five, the immune system-potentiating amount is a human immune system-potentiating amount, and said pharmaceutical dosage unit is from about 900 to about 1200 mg/m^2 body surface area of said human.

13. The composition of Claim 7, wherein said interferon is an αinterferon and said amount is between about 1 million and about 3 million units of said interferon.

14. The composition of Claim 7, wherein said thymosin is Thymosin α-1, said immune system-potentiating amount is a human immune system-potentiating amount, and said pharmaceutical dosage unit is from about 900 to about 1200 μg/m^2 body surface area of said human.

15. The composition of Claim 7, wherein said thymosin is Thymosin α-1, and said pharmaceutical dosage unit contains about 1500 to about 1700 μg of Thymosin α-1.

16. An anti-hepatitis C formulation comprising an immune sytem-potentiating amount of at least one thymosin or an immune system-potentiating thymosin fragment in combination with an anti-viral effective

amount of at least one interferon in a pharmaceutically acceptable carrier, for use in the treatment of a mammal infected with hepatitis C virus.

17. The formulation of claim 16, wherein said thymosin is selected from the group consisting of Thymosin Fraction Five and Thymosin α-1.

18. The formulationof Claim 16, wherein said interferon is selected from the group consisting of α-, β-, and γ-interferons.

19. The formulation of Claim 18, wherein said α-interferon is interferon α-2B.

20. The formulation of Claim 19, wherein said interferon is recombinant interferon.

21. The forumlation of Claim 16, wherein said thymosin is Thymosin Fraction Five, said immune system-potentiating amount is a human immune system-potentiating amount, and said amount is from about 900 to about 1200 mg/m^2 body surface area of said human.

22. The formulation of Claim 16, wherein said interferon is α-interferon and wherein said anti-viral effective amount is from about 1 million to about 3 million units of said interferon.

23. The formulation of Claim 16, wherein said thymosin is Thymosin α-1, said immune system-potentiating amount is a human immune system-

potentiating amount, and said amount is from about 900 to about 1200 $\mu g/m^2$ body surface area of said human.

24. The formulation of Claim 16, wherein said thymosin is Thuymosin α-1, and wherein said amount is about 1500 to about 1700 μg of Thymosin α-1.

ABSTRACT

Compositions and methods of use for treating hepatitis C virus-infected mammals are disclosed. The compositions include one or more thymosins in combination with one or more interferons. Methods of treatment include use of thymosins together, or sequentially with interferon.

DECLARATION FOR PATENT APPLICATION

Docket No. ____________

As a below named inventor, I hereby decla   iat:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled ____________, the specification of which

Composition and Method of Treating Hepatitis C

APR 0 6 2000

PATENT & TRADEMARK OFFICE

(check one) ☐ is attached hereto.
☒ was filed on May 4, 1992 ____________ as
Application Serial No. 07/878,372
and was amended on ____________ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status—patented, pending, abandoned)
07/759,544	Sept. 13, 1991	Pending

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Anthony T. Lane, Reg. No. 19,964 and Werten F. W. Bellamy, Reg. No. 27,029

Address all telephone calls to Werten F.W. Bellamy at telephone no. 703-696-8119

Address all correspondence to Werten F.W. Bellamy 703-696-8119
Intellectual Property Law Division
Office of The Judge Advocate General, DA
901 N. Stuart Street, Suite 400
Arlington, VA 22203-1837

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Kenneth Eliot Sherman

Inventor's signature Kenneth Eliot Sherman Date 30 July 1992

Residence 5557 S. Yampa Street Citizenship USA

Post Office Address Aurora, Colorado 80015

Full name of second joint inventor, if any ____________

Second Inventor's signature ____________ Date ____________

Residence ____________ Citizenship ____________

Post Office Address ____________

(Supply similar information and signature for third and subsequent joint inventors.)

Form PTO-FB-A110 (8-83)

Please type a plus sign (+) inside this box → [+]

PTO/SB/122 (11-96)
Approved for use through 6/30/99. OMB 0651-0035
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

CHANGE OF CORRESPONDENCE ADDRESS

Application

Address to:
Assistant Commissioner for Patents
Washington, D.C. 20231

Application Number	08/844/349
Filing Date	
First Named Inventor	Kenneth E. Sherman
Group Art Unit	1643
Examiner Name	Jay Williams
Attorney Docket Number	0100

Please change the Correspondence Address for the above-identified application to:

[ ] Customer Number [ ] → *Type Customer Number here*

OIPE APR 0 6 2000 PATENT & TRADEMARK OFFICE

Place Customer Number Bar Code Label here

OR

[X] Firm *or* Individual Name	Maria H. Sjogren, M.D., MC, Col. U.S.A.				
Address	Chief, Department of Clinical Investigation, WRAMC,				
Address	Bldg.6, Rm. 4018, 6900 Georgia Avenue; N.W.				
City	Washington	State	D.C.	ZIP	20307
Country	U.S.A.				
Telephone	(202) 782-7859	Fax	(202) 782-3881		

This form cannot be used to change the data associated with a Customer Number. To change the data associated with an existing Customer Number use "Request for Customer Number Data Change" (PTO/SB/124).

I am the :

[ ] Applicant.

[ ] Assignee of record of the entire interest.
Certificate under 37 CFR 3.73(b) is enclosed.

[X] Attorney or agent of record .

Typed or Printed Name	Werten F.W. Bellamy
Signature	Werten F.W. Bell
Date	April 6, 2000

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.